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Abbreviations

AZA: Azathioprine

CSA: Cyclosporine A

DXM: Dexamethasone

GCs: Glucocorticoids

GM: Gut microbiota

GUS: B-glucuronidase

IST: Immunosuppressive treatment

KT : Kidney transplantation

LPS : Lipopolysaccharide

MLC2: Myosin light chain 2

MLCK: Myosin light chain kinase

MMF: Mycophenolate mofetil

MPA: Mycophenolic acid

mTOR: Mammalian target of rapamycin

PCR: Polymerase chain reaction

SOT: Solid organ transplant

TNF: Tumor necrosis factor

UTI: Urinary tract infection

Abstract

Our understanding of the involvement of the gut microbiota (GM) in human health has expanded exponentially over the last decades, particularly in the fields of metabolism, inflammation and immunology. Immunosuppressive treatment (IST) prescribed to solid organ transplant (SOT) recipients produce GM changes that affect these different processes. This review aims at describing the current knowledge of how IST change the GM.

Overall, SOT followed by IST results in persistent changes in the GM, with a consistent increase in proteobacteria including opportunistic pathobionts. In mice, Tacrolimus induces dysbiosis, metabolic disorders, and alters the intestinal barrier. The transfer of the GM from Tacrolimus-treated hosts confers immunosuppressive properties, suggesting a contributary role for the GM in this drug's efficacy. Steroids induce a dysbiosis and intestinal barrier alterations, and also seem to depend partly on the GM for their immunosuppressive and metabolic effects. Mycophenolate Mofetil, frequently responsible for digestive side effects such as diarrhea and colitis, is associated with pro-inflammatory dysbiosis and increased endotoxemia. Alemtuzumab, m-TOR inhibitors and belatacept have shown more marginal impacts on the GM.

Most of these observations are descriptive. Future studies should explore the underlying mechanism of IST-induced dysbiosis in order to better understand their efficacy and safety characteristics.

Key words: immunosuppressive drugs, gut microbiota, side effect, bacteria, solid organ transplantation

1. The gut microbiota in solid organ transplantation: why should we care?

The gut microbiota (GM) is the total microbial mass that colonizes the digestive tract of a host. It is composed of trillions of bacteria, viruses, archaea, fungi that are distributed throughout the digestive tract with different abundances[1,2]. Our knowledge of the GM has dramatically increased over the last decades principally because of the development of new genetic tools[3].

Solid organ transplantation can only be considered medically when accompanied by the long-term use of immunosuppressive treatments (IST), to avoid graft rejection. However, ISTs are responsible for major side effects such as infections, diabetes and high blood pressure.

Yet, transplantation and ISTs prescribed after transplantation have been shown to be associated with changes in the GM. The GM has a wide array of functions that benefit the host, such as digestion and energy harvesting[4], protection from colonization by pathogens[5], training of the immune system[6], fat storage[7], neuropsychological development[8], homeostasis[9] and the metabolism of xenobiotics[10]. The GM is capable of resilience, *i.e.* it has the ability to restore a homeostatic balance between the different sub-populations despite external perturbations[11]. Durable modifications of the GM, called dysbiosis, have been associated with several chronic diseases, such as cardiovascular (high blood pressure[12–15]), metabolic (diabetes mellitus[16–19] and obesity[11]) and neurological disorders[20,21], and inflammatory bowel diseases[22–24].

It has been demonstrated that some drugs used in human health interact with the GM. On the one hand, treatments with antibiotics[25–27], chemotherapies[28–31] and many others modify the microbiota, which can lead to dysbiosis and adverse events such as obesity[32]. On the other

hand, the anti-diabetes drug metformine[33] and checkpoint blocker ipilimumab[34] exert their beneficial effects by modifying the GM, which in turns affects the host physiology.

According to the Global Observatory on Donation and Transplantation, more than 130,000 solid organ transplantations have been performed in 2016, including nearly 90,000 (69%) kidney transplantations[35], exposing recipients to prolonged ISTs to avoid graft rejection[36].

1.1. Bidirectional interactions between the gut microbiota and the host immunology

The GM interacts with host immunity through a complex crosstalk. The involvement of the GM in the maturation of the immune system was demonstrated by the study of axenic (*i.e.* germ-free) mice, which develop an immature immune system with atrophic lymphatic nodes, spleen and Peyer's patches[37].

Innate immunity is generally affected as well. Microbial peptidoglycans and flagellins induce the production of anti-microbial peptides by intestinal epithelial cells in the lumen of the digestive tract. The buildup of this immunity involves the transcription factor NF-κB and the Toll-like Receptor 5 of dendritic cells, respectively[38]. The GM also impacts adaptative immunity, such as the inflammation/tolerance balance (Th1-2-17/T_{reg}s-Th1)[39]. As an example, *Bacteroides fragilis* –one of the anti-inflammatory members of the GM –induces T_{reg} lymphocytes and attenuates colitis[40]. The polysaccharide A at the surface of *Bacteroides fragilis* is recognized by the TLR2 receptor of $CD4^+$ cells[41], resulting in their differentiation into T_{reg} lymphocytes. T_{reg}s repress the pro-inflammatory activity of Th17 cells through the local and systemic secretion of interleukin 10[41].

This GM-driven redirection of the inflammation/tolerance balance has clinical consequences. For example, a pro-inflammatory GM may be involved in the progression of chronic kidney

disease[42,43]; in a 'vicious circle', uremic toxins modify the microbial composition of the gut, which promotes systemic inflammation and the further progression of kidney lesions. Conversely, the host immune system controls the GM; when immunosuppressed mice are colonized with opportunistic pathogens[44], they develop septicemia with digestive commensals[45].

The importance of the bidirectional interactions between the GM and the host's immunity needs to be explored in the specific context of organ transplantation and IST. If the host's immune system controls the GM, what is the impact on the GM of drugs which interfere with the immune system?

1.2. Modifications of the GM after liver transplantation

Zhong When-Wu *et al*. studied the fecal microbiota of liver transplant recipients, as compared to healthy volunteers and cirrhotic patients[46]. They highlighted that liver transplantation was associated with gut dysbiosis characterized by a decrease of the total bacterial mass, and of beneficial bacteria such as *Bifidobacterium spp.*, *Faecalibacterium prausnitzii* and *Lactobacillus spp.* and an increase of *Enterobacteriaceae* and *Enterococcus spp*. However, 13-24 months after liver transplantation, all bacteria populations tended to return to a normal level, except for *Enterococcus spp*. which was responsible for infections (urinary tract, surgical site and bacteremia) after the liver transplantation[47].

1.3. Modifications of the GM after kidney transplantation

1.3.1. Description of the GM alterations after kidney transplantation

Fricke *et al.*[48] studied the evolution of the gut, urine and oral microbiotas after kidney transplantation (KT) in 60 patients. They observed an early switch in the GM composition after KT. Modifications were more extensive between before KT and one month after KT

than between 1 month and 6-month after KT, and persisted over time, suggesting a loss of the GM resilience.

In another study, Bacteroidetes relative abundance appeared to be reduced in KT recipients, while Proteobacteria were increased[49]. The Proteobacteria phylum contains opportunistic pathogens such as *Salmonellae*, and *Escherichia coli*, and is considered a signature of an unstable and pro-inflammatory GM[50].

1.3.2. Adverse events after kidney transplant correlate with gut microbiota modifications

1.3.2.1. Pre-transplant gut microbiota is associated with post-transplant clinical events

In the study of Fricke *et al.*[48], those transplant patients who experienced rejection had, prior to transplantation, a significant scarcity of four bacterial genera from the Firmicutes phylum (*Anaerotruncus, Coprobacillus, Coprococcus* and an unknown member of the Peptostreptococcaceae), compared to patients without rejection events. *Anaerotruncus* was also significantly depleted prior to KT in the GM of patients who experienced post-transplantation infection.

Finally, in our recent study, patients who developed New Onset Diabetes After (kidney) Transplantation tended to have a higher carriage of fecal *Lactobacillus sp.* prior to KT, and also had a lower abundance of *F. prausnitzii* than patients who did not develop diabetes[51].

1.3.2.2. Post-transplant gut microbiota differences are associated with post-transplant clinical events

Lee *et al.*[52] described a link between important post-KT complications (*i.e.* diarrhea, rejection, and urinary tract infection, or UTI) and GM modifications. Patients who experienced post-transplant diarrhea carried a decreased bacterial diversity as measured by the Shannon Index

and gut dysbiosis, characterized by a lower relative abundance of the *Bacteroides, Coprococcus*, *Ruminococcus*, *Dorea* genera. Patients who experienced acute rejection had a higher relative abundance of Lactobacillales, *Enterococcus*, *Anaerofilum*, and *Clostridium tertium*, and a lower relative abundance of Clostridiales, Bacteroidales, and Lachnospiraceae and of *Blautia*, *Eubacterium dolichum*, *Ruminococcus*, and *Bacteroides*. Finally, patients who suffered from UTI after KT had a higher relative abundance of *Enterococci* in their feces.

These studies seem to indicate that a low abundance of bacteria belonging to the Clostridiales order (*Anaerotruncus*, *Coprococcus*, *Ruminococcus*, *Dorea, Eubacterium Dolichum, Lachnospiraceae, Blautia,* and *F. prausnitzii*) is associated with adverse events after KT. Interestingly, some of these bacteria are producers of short chain fatty acids, which are considered beneficial for the host[53].

It is important to note that the causality of these associations has not been investigated. Yet, these studies suggest that the GM is modified after solid organ transplantation (SOT) and IST and raise the question of the implication of both pre- and post-transplant microbiota in the onset of the main post-transplant complications. Therefore, a better understanding of GM modifications after SOT/IST appears to be required.

SOT is associated with many factors possibly involved in the modification of the GM, such as anti-infectious prophylaxis, general anesthesia, dietary changes, and the restoration of the transplanted organ function (**Figure 1**). Recently, several studies have demonstrated that IST also modified the GM, and this review focuses on this aspect.

2. Corticosteroids

Glucocorticosteroids (GCs) remain a major part of anti-rejection treatment strategies following a SOT[54].

2.1. Gut microbiota changes induced by corticoid treatment in rodents

In a mouse model, glucocorticoids decreased bacterial richness and diversity (Chao1 and Shannon indexes)[55] and altered the global composition of the GM[56].

At the phylum level, the literature is heterogeneous. Our study showed an increased Firmicutes/Bacteroidetes ratio[56], while other studies found a decrease of Firmicutes[55], Bacteroidetes, Actinobacteria, alpha and gamma Proteobacteria[55] and Deferribacteres[57].

At deeper levels, GCs were reported to increase the relative abundance of fecal Clostridiales, *Lactobacillus*[55]*, Anaerostipes*[57], *Bifidobacterium*[58] and decreased *Oscillospira, Bilophila,* and *Rikenella*[57]. In addition, two studies found a decrease of *Mucispillirum*[54,55], mucin degrading bacteria[59] that are involved in the maturation and the activation of T cells through an interaction with antigen presenting cells[60].

The GM is physiologically capable of resilience, defined as its ability to return to an initial state after a disturbance[61]. Kim *et al*. demonstrated that dexamethasone (DXM) administration in mice increased the delay before GM demonstrated resilience, exposing mice to more severe *Clostridium difficile* infection[62]. Finally, GCs decreases *Clostridium sensu stricto* abundance in the ileum[56].

2.2. Disruption of circadian rhythms as a potential mechanism of GC-induced dysbiosis

Many organisms undergo 24 hours cycles, with markers varying in a circadian rhythm[63]. Similarly, the GM exhibits daily fluctuations and any perturbations of the circadian rhythm

that the host experiences (such as jetlag) is responsible for a loss of rhythmicity associated with dysbiosis[64]. Interestingly, endogenous glucocorticoids regulate the circadian rhythmicity[65] and exogenous GCs such as dexamethasone are strong perturbators of circadian rhythm-related genes, thus counteracting endogenous steroids[66]. Wu *et al.*[55] studied the effect of chronic administration of GCs on the circadian rhythm and the GM. After seven weeks of treatment, rats exhibited the following changes: alteration of the global composition of the GM characterized by a decreased richness and diversity, a decrease of the main phyla as described in the preceding section, attenuation of the weight gain, an accumulation of fat and loss of their circadian rhythm.

2.3. Glucocorticoids alter the intestinal barrier

The intestinal barrier is composed of three layers: the lamina propria, epithelial intestinal cells, and the mucus layer. The mucus layer plays a major role in pathogen resistance[67], by the expression of virulence genes of pathogens and in the nutrition of the GM[68]. The intestinal barrier maintains a physiological distance between the GM and the body by chemical, mechanical and immunological means and also participates in the regulation of the GM[69,70].

Treatment with DXM is responsible for a decreased expression of Muc2, the main component of the colonic mucus[55,58]. Interestingly, Muc2 expression was not altered when DXM was given to germ-free mice, suggesting that downregulation of Muc2 by DXM implicates the GM. Conversely, fecal microbiota transplantation from DXM-treated mice led to an increased expression of Muc2 and a reduced inflammation in recipient mice that were genetically susceptible to colitis (IL-$10^{-/-}$ knockouts).

GCs also alters the innate immunity of the intestinal barrier. Indeed, prednisolone was responsible for a decreased expression of C-type Lectins RegIIIβ and RegIIIɣ[56], which are

involved in the control of intestinal bacterial proliferation and the host's immune response to the GM[71]. Moreover, administration of DXM to rats decreased the level of biliary IgA[72] and decreased IgA bacterial coating[73], a process that neutralizes pathogenic bacteria[74]**.** Finally, an *in vitro* study showed that GCs prevented a TNF-α-induced increase in intestinal epithelial permeability. The binding of GCs on their receptor inhibited TNF-α-induced expression of Myosin Light Chain Kinase (MLCK). MLCK phosphorylates and activates Myosin Light Chain 2 (MLC2), which is responsible for the contraction of the peri-junctional actin-myosin filaments, leading to the dysfunction of the tight junctions. As a consequence, GCs inhibited TNFα-induced tight junction dysfunction[75] (see **Figure 2** and **Table 1** for an overview of the effects of the various ISTs on the GM discussed in this Review).

2.4. The gut microbiota is involved in GC metabolism and efficacy

In the gut, endogenous GCs are metabolized by *Clostridium scindens*[76]. In a large pharmacological study, Zimmermann *et al.* showed that many drugs could be metabolized by specific members of the GM; in particular, DXM, prednisone, prednisolone, cortisone and cortisol were metabolized by *C. scindens* and *Propionimicrobium lymphophilum* into androgens. Mice colonized with *C. scindens* exhibited lower concentrations of DXM in their caecum and higher concentrations of androgens than germ-free mice[77]. Because androgens (and estrogens) are also metabolized by some gut microbiota members (*Steroidobacter denitrificans*[78] and *Comamonas testosterone*[79]), the net clinical consequences of these processes on the intestinal androgens are challenging to investigate. Among the hypothetical consequences of an increase in androgens due to GM-metabolized prednisone are: prostate cancer[80], bowel disorders and mood changes[81].

Finally, in a mouse model of lupus, He *et al.* demonstrated that an improvement of lupus was associated with prednisone-induced GM modifications[57]. These included decreases in *Mucispirillum*, *Oscillospira*, *Bilophila* and *Rikenella* populations, and an increase in *Anaerostipes*.

In conclusion, GCs are responsible, at least in part, for the modification of the intestinal barrier. The mechanisms underlying GC-induced GM modifications need further investigation but could involve the disruption of the circadian clock, and the modulation of the immunology of the intestinal barrier. GM also appears to be an actor of the metabolism of GCs (**Figure 2**).

3. Calcineurin inhibitors

3.1. Tacrolimus

Tacrolimus is a macrolide that was first developed as an antibiotic before the discovery of its immunosuppressive properties. Tacrolimus is a calcineurin inhibitor that binds to the FK506-binding protein to form a complex that inhibits calcineurin phosphatase[82]. For over ten years it has been the cornerstone of anti-rejection treatment in kidney transplantation[83]. Despite its reliable efficacy in maintaining immunosuppression and avoiding graft rejection, tacrolimus exhibits major metabolic side effects including glucose intolerance, diabetes[84] and high blood pressure[85].

3.1.1. Tacrolimus modifies the gut microbiota

During the past five years, several studies have demonstrated an impact of tacrolimus on the rodent fecal microbiota (**Figure 3**). While these studies consistently identified changes in the GM, the characteristics of the changes diverge.

When given by oral gavage, tacrolimus did not alter the richness (alpha diversity) the Firmicutes/Bacteroidetes ratio[56,86,87], neither in rats nor in mice. However, Toral *et al.* found that intraperitoneal injection of tacrolimus in a mouse model was responsible for a decrease of microbial diversity as measured by the Shannon Index, and an increase of the Firmicutes/ Bacteroidetes ratio[88].

In rats, intraperitoneal tacrolimus decreased the fecal relative abundance of Mollicutes, Micrococcaceae, Actinomycetales*, Roseburia*, *Oscillospira, Rothia,* and *Staphylococcus* and increased *A. muciniphila*[86]. In mice, oral tacrolimus decreased Ruminococcaceae (of which *Oscillospira* and *Ruminococcus*[87]*)*, *Clostridium*, *Rikenella*, and *Bifidobacterium*[88] and increased the abundance of *Allobaculum*, *Bacteroides*, *Lactobacillus* and *A. muciniphila*.

These variations of the intestinal microbiota are relevant for several reasons: first, *Allobaculum* was found to be increased in a model of immunodeficient mice[89] and might be a "gut signature" of tacrolimus-induced immunosuppression. Second, tacrolimus triggers a gut dysbiosis that is analogous to that observed in metabolic diseases, *i.e*. an increased Firmicutes/Bacteroidetes ratio that is a major marker of the gut dysbiosis linked to metabolic diseases[90–92]. Moreover, tacrolimus seems to have a depleting effect on short-chain fatty acid producing bacteria such as *Ruminococcus spp.* and *Bifidobacterium*. Interestingly, acetate and butyrate are involved in the regulation of blood pressure[93], lipogenesis and blood glucose[94,95]. *Oscillospira*, also depleted in tacrolimus-treated rats and mice, is associated with a "metabolic-friendly" microbiota[96]. Finally, tacrolimus-induced gut dysbiosis shares similarities with a type 2 diabetes GM that is enriched in *A .muciniphila* and depleted in *Roseburia*[95].

However, it is possible that tacrolimus has different impacts on the GM depending on the dose that was administered. In a rat liver transplant model, an intermediate dose (0.5 mg/kg of body mass) of tacrolimus was associated with an increase of microbial richness and of the beneficial bacteria *Bifidobacterium* and *F. prausnitzii,* and a decrease of less beneficial bacteria such as *Enterobacteriaceae* and *Bacteroides-Prevotella*. These changes were associated with a better liver transplant outcome. In contrast, lower (0.1 mg/kg) and higher (1 mg/kg) doses of tacrolimus were associated with better microbial richness, and an increased abundance of *Enterobacteriaceae* and decrease of *Bifidobacterium* and *F. prausnitzii.* Both high and low doses of tacrolimus were associated with a poorer allograft outcome and endotoxinemia[97].

3.1.2. Tacrolimus alters the GM metabolic functions

Using PICRUST analysis, a bioinformatic tool that predicts the functional composition of the GM using marker gene data[98], tacrolimus was found to alter functions of the GM such as lipid and carbohydrate metabolism[87]. These results were confirmed by metagenomic analysis[86]. Tacrolimus altered carbohydrate metabolism and starch degradation by inducing a shift from a mostly anabolic microbiota towards a catabolic one[86].

Interestingly, modulation of the GM with a strain of *Lactobacillus* that is used as a probiotic prevented tacrolimus-induced hyperglycemia in rats[86]. Toral *et al*.[88] showed that fecal microbiota transplantation from tacrolimus-treated mice induced high blood pressure in the recipients. Another strain of *Lactobacillus* (LC40) also corrected tacrolimus-induced high blood pressure and endothelial dysfunction.

3.1.3. Tacrolimus alters the intestinal barrier and modulates immunity

Tacrolimus increases intestinal permeability in a dose-dependent manner (**Figure 3**). This could be a consequence of a decreased expression of occludin and Muc3 in the colon[88] and partly responsible for the observed endotoxinemia[97]. Moreover, tacrolimus is responsible for a local immunosuppression in the gut by inhibiting mucosal T-lymphocyte and NK cells functions[99]. Tacrolimus also decreases RegIIIβ and RegIIIɣ expression[56], two lectins (antimicrobial peptides secreted by the host as a response to IL-22) that regulate bacterial density in the GM and are involved in the host's innate immunity response to the GM[71]. This in turns possibly modifies the GM[56].

Interestingly, either tacrolimus treatment or the transfer of the fecal microbiota from tacrolimus-treated mice resulted in an increase of the proportion of $CD4^+$ $CD25^{hi}$ $FoxP3^+$ regulatory T cells in the colonic mucosa of recipient mice, as well as the circulation[87]. This is a clear indication that the effect of tacrolimus on regulatory T cells is mediated through the microbiota.

Using a skin transplant model, Zhang *et al*. demonstrated that fecal transfer from tacrolimus-treated mice elicited immunosuppressive properties, and improved skin allograft survival in the recipient when combined with a low-dose of tacrolimus, administered by gavage[87].

3.1.4. Gut microbiota: an actor of tacrolimus metabolism

A pilot study[100] has shown that patients who require high doses of tacrolimus to reach the target trough level (plasma concentration) harbored a higher relative abundance of *F. prausnitzii* in their GM. Moreover, *F. prausnitzii* abundance at one week after KT was positively correlated with future dosing of tacrolimus at one month. This study provides evidence for a new pathway of tacrolimus metabolism, even if some confounding parameters were not taken into account (such as patients dietary habits that modulate both tacrolimus

absorption[101] and *F. prausnitzii* abundance, or CYP3A5 gene polymorphism, which is known to modulate FK pharmacokinetics[102]) this study possibly evidences a new pathway for tacrolimus metabolism.

The same authors[103] showed that some commensal gut bacteria such as *F. prausnitzii* or *Clostridiales* were able to transform tacrolimus into a 15-fold less active metabolite *in vitro*.

To summarize, tacrolimus alters the GM composition which affects the host. The GM of tacrolimus-treated mice seems to have immunosuppressive properties. Even if the results are not always consistent, tacrolimus appears to deplete bacteria that positively affect their host's metabolism. These results question the role of the GM in tacrolimus' immunosuppressive activity and side effects. An additional level of complexity comes from the observation that the response of the GM may differ depending on the dose of tacrolimus. Conversely, the GM could be involved in tacrolimus metabolism and explain part of the inter-patient variability of tacrolimus dosing.

3.2. Cyclosporine

Cyclosporine A (CSA) is another calcineurin inhibitor used in SOTs. It selectively inhibits T-lymphocyte activation[104]. Jia *et al.* studied the impact of CSA on the GM in a rat model of allogenic liver transplantation[105]. The GM was explored by denaturing gradient gel electrophoresis and by the quantification of specific bacteria using PCR. CSA increased the GM richness with an enrichment of *F. prausnitzii* compared to the control group (allograft without CSA). On the contrary, CSA decreased the proportion of Enterobacteriaceae and of *Clostridium* clusters I and XIV. Interestingly, the analysis of the GM gel electrophoresis profiles clustered the non-rejecting rats (sham surgery, isograft and CSA-treated allograft) as

opposed to the rejecting rats (non-treated allograft). This indicates that CSA tends to restore the pre-transplant GM.

4. Anti-metabolites

4.1. Mycophenolate Mofetil

Mycophenolate Mofetil (MMF) is a prodrug that is converted into mycophenolic acid (MPA), which inhibits purine synthesis through the inhibition of inosine monophosphate dehydrogenase (**Figure 4**). After intestinal uptake MPA is glucuronidated by a liver enzyme, leading to its inactivation. The inactive form of MPA is then eliminated by the bile and the stool[106].

It is the main drug responsible for digestive disorders after KT, affecting 30 to 50% of all MMF-treated patients[107] with symptoms that include diarrhea, abdominal pain, vomiting, and, rarely, digestive ulcer or haemorrhage[107]. Endoscopic and histological patterns of MMF-induced colitis can mimic acute colitis[108]. This major side effect can lead to an impaired quality of life and an irregular observance, increasing the risk for allograft rejection[109]

The pathophysiology of MMF-induced colitis has recently been explored and there is evidence for a potential role of the GM.

4.1.1. MMF induces gut microbiota modification and endotoxemia.

Mice treated with MMF exhibited significant markers of dysbiosis, such as an early and persistent diminution of microbial richness, an increase of the Firmicutes/Bacteroidetes ratio and a relative abundance of *Clostridia* and *Bacteroides spp*.[110] and of Proteobacteria, including the opportunistic pathogens *Escherichia* and *Shigella*. In contrast *Akkermansia*, *Parabacteroides* and *Clostridium* were decreased[111].

These abnormalities were associated with an increase of circulating lipopolysaccharide (LPS) , a major cell wall component of Gram-negative bacteria. Endotoxemia leads to low-grade inflammation that in turn promotes metabolic syndrome and insulin resistance in rodents[112], and cardiovascular events in KT recipients[113]. This increased endotoxemia is attributed to the increase of Gram-negative bacteria in the GM and an alteration of gut tight junction induced by MPA, resulting in increased gut permeability[114].

4.1.2. MMF-induced colitis is associated with dysbiosis.

In conventionally raised mice, MMF induces cecal atrophy, colitis and weight loss[110]. When MMF was administered to axenic mice, or mice previously treated with broad-spectrum antibiotics, they did not develop these disorders, suggesting that MMF induces a dysbiosis that is responsible for the digestive disorders.

Taylor *et al*. showed that MMF was responsible for an increase of *Clostridia*, and *Bacteroides spp*. in mice[110]. They observed a simultaneous rise in gene expression and activity of gut B-glucuronidase (GUS) in the caecum and the colon. This enzyme, which is expressed by *Bacteroides*, converts the inactive glucuronidated MPA into its free active form. Interestingly, addition of vancomycin was responsible for a decrease of *Bacteroides*, of GUS activity and of free MPA in mice's stools. The use of vancomycin also abrogated MMF gastro-intestinal side effects such as weight loss, cecal atrophy and colonic inflammation. Moreover, in eleven human patients, the author found that GUS activity correlated with MMF exposure. Finally, intrarectal infusion of MPA was responsible for weight loss in mice. Altogether, these results suggest that MMF promotes *Bacteroides* proliferation in the gut of mice, which results in an increased GUS activity and therefore an increase of colitogenic free MPA in the colon.

These two studies therefore provide interesting insights into the pathophysiology of MMF-induced gastro-intestinal side effects.

4.2. Azathioprine

Azathioprine (AZA) is another anti-metabolite that has been used for a long time in kidney transplants[115]. It is the prodrug of 6-mercaptopurine, a purine antagonist which inhibits DNA synthesis[116,117].

AZA was found to inhibit the proliferation of some enteric bacteria *in vitr*o: *Campylobacter concisus*, *Bacteroides fragilis*, and *Bacteroides vulgatus*[118]. Only the highest concentration of AZA (200 µg/ml) inhibited the growth of *E. coli*. AZA did not significantly affect the growth of *E. faecalis*. In a cohort of 20 patients with inflammatory bowel diseases, AZA was found to increase the concentration of mucosal bacteria compared to healthy controls, and the percentage of the epithelial surface covered with adherent bacteria compared to patients with inflammatory bowel diseases[119].

5. mTOR inhibitors

Rapamycin and everolimus are mammalian target of rapamycin (mTOR) inhibitors which interfere with lymphocyte proliferation[120] by inhibiting mTOR complex 1 and 2. They are frequently used in SOT and are responsible for metabolic, infectious and hematologic adverse events[121]. Recent studies have highlighted the impact of this therapeutic class on the GM.

5.1. Rapamycin and everolimus change gut microbiota composition

Everolimus seems to have little impact on the GM[56]. The global composition was not significantly altered in mice treated with this drug as compared to controls as evaluated by principal component analysis. Rapamycin decreased the phylogenetic diversity score in rats[86],

and influenced beta diversity but not alpha diversity in mice[122]. Specifically, rapamycin affected gut dysbiosis induced by a high fat diet *i.e.* it changed the relative abundance of *Turicibacter,* unclassified Marinilabiliaceae*, Alloprevotella,* unclassified Porphyromonadaceae*, Ruminococcus, Bifidobacterium, Marvinbryantia, Ruminococcus* (Lachnospiraceae)*, Helicobacter,* and *Coprobacillus.* In a rat model, rapamycin decreased the bacterial diversity and altered beta diversity composition with a decrease of *Roseburia, Oscillospira, Mollicutes, Rothia, Micrococcaceae, Actinomycetales, and Staphylococcus*[86]. Using a *Drosophila* model, Schinaman et *al.* found that rapamycin treatment was associated with alteration of the gut microbiota, namely a reduction of bacterial mass and decrease of Alphaproteobacteria[123]

5.2. Effect of mTOR inhibition on gut barrier function and immune and metabolic disorders.

Intestinal cell differentiation and proliferation are regulated by mTOR Complex 1, in a Notch-dependent manner. The excessive activation of this pathway alters goblet and Paneth cell differentiation[6,124]. This stimulation is attenuated by rapamycin[125]. Paneth cells, located in the small intestinal mucosa, play a central role in the crosstalk between the innate immune system and the GM through the synthesis of antimicrobial peptides. We showed that, in mice, everolimus decreases the ileal expression of IL-22 and consequently of C-type lectins RegIIIβ and RegIIIɣ[56]. The mTOR pathway is an important actor in intestinal epithelial cell proliferation, through Wnt signaling. Therefore, inhibition of mTOR interferes with enterocyte proliferation[126]. In a murine model, Xie et *al.* found that hyperactivation of intestinal mTOR induced by a Western diet was responsible for an increased necroptosis of intestinal epithelial cells, and a marked susceptibility to develop colitis[127]. It was associated

with gut barrier dysfunction, measured by an increased intestinal permeability (an increased blood translocation of FITC-Dextran given by gavage). Rapamycin prevented colitis and reduced inflammation. Furthermore, GM depletion by antibiotics attenuates mTOR hyperactivation associated with intestinal disorders, further suggesting that the GM is involved in mTOR hyperactivation. [127]

Finally, Rapamycin-induced gut dysbiosis correlated with metabolic disorders such as body weight increase, insulin resistance, intestinal inflammation (measured through fecal lipocalin-2) and fat deposition in mice receiving a high fat diet[122]. Peyer patch immune modifications (increases in $IL17^+$ $CD4^+$ T cells) induced by rapamycin correlated with GM modifications[128].

6. Belatacept

In 2011, belatacept was approved as a maintenance immunosuppressive drug after kidney transplantation in 2011 and shows better patient and graft survival, and better kidney function in the long term compared to CSA based regimens[129]. It blocks T-cell activation by inhibiting the co-stimulation signal[130].

No data regarding the impact of this treatment on the GM has been published so far. However, a recent case report relates the occurrence of severe intestinal lesions (Crohn-like colitis) induced by belatacept[131]. The possible role of the GM in this complication needs to be explored.

7. Alemtuzumab

Alemtuzumab is a monoclonal antibody that binds to the CD52 antigen, thereby depleting T and B-lymphocytes[132]. In KTs, alemtuzumab is used for induction, and efficiently reduces post-transplant acute rejection[133].

7.1. Alemtuzumab alters the GM and mucosal immunity

In addition to its depleting effect on circulating lymphocytes, alemtuzumab also depleted and altered the function of intestinal mucosa lymphocytes in a monkey model[134]. This depletion of T-lymphocytes in intestinal mucosa was associated with a shift in the GM composition. In ileal mucosa, alemtuzumab was responsible for an increase of *Prevotella*, and a higher proportion of opportunistic pathogenic bacteria such as *E. coli, Shigella Flexneri*. In the fecal microbiota, alemtuzumab was responsible for an increase of Clostridiales and a decrease of *F. Prausnitzii*. Interestingly, microbial modifications were restored 35 days after alemtuzumab discontinuation and mucosal lymphocyte regeneration.

7.2. Alemtuzumab alters the fungal microbiota.

Mucosal lymphopenia induced by alemtuzumab was also associated with fungal microbiota dysbiosis in a cynomolgus monkey model[135]. There was an increased diversity and colonization by *Candida albicans*, *Aspergillus clavatus*, and *Saccharomyces cerevisiae*. Interestingly, resilience of the fungal microbiota was observed in this study, as the fungal microbiota of colonic mucosa was restored concomitantly with T lymphocyte reconstitution. Alemtuzumab treatment decreased the diversity of the fecal fungal microbiota, and increased *Candida albicans*, *Saccharomyces cerevisiae* and *Botryotinia fuckeliana*.

8. Combined therapy

Very few studies have investigated the impact of simultaneous multiple IST on the GM (**Figure 5**) even though this is clinically relevant, because ISTs in transplantation are in fact usually combined[136], as per recommendations[137].

8.1. Rodent studies

We have shown that a combined therapy including prednisolone, tacrolimus and MMF depleted *Clostridium sensu stricto* in the ileum. The combined therapy also altered the GM, by modifying the global composition of the fecal microbiota[56], favoring the proliferation of both commensal and uropathogenic *E.coli*. We hypothesized that this microbiological side effect could participate in the high prevalence of urinary tract infections after KT[138].

It is possible that the decreased secretion of innate ileal anti-microbials (lectins RegIIIɣ and RegIIIβ) participated in these modifications. Lectins were also decreased after treatment with prednisolone and tacrolimus separately, but no synergistic action of the combined treatment was observed compared to monotherapies.

8.2. Human studies.

In a study that included a small number of patients, Zaza *et al*. have demonstrated that KT recipients treated with tacrolimus and MMF exhibited a different gut composition in bacterial genes categorized by function than patients treated with everolimus and MMF[139]. Treatment with tacrolimus was associated with a decrease in the genes coding for the macrolide transport system (mrsA) and an enrichment in genes coding for flagellar motor switch proteins and type IV pilus assembly proteins. These proteins are expressed by *Enterobacteriaceae* and the bulk enrichment in genes coding for the flagellar apparatus could be the consequence of their proliferation under immunosuppressed conditions. Thus, this study suggests the development of a specific gut signature in IST-treated patients with specific functional characteristics, the clinical implication of which still have to be explored.

9. Direct and indirect alterations of the GM by IST

It appears that there are bidirectional interactions between the GM and the IST-modified host immunity. A key question is to decipher the underlying mechanisms of this crosstalk. This an

extremely arduous task as there are many “speakers” partaking in this dialogue. The GM communicates through metabolites and antigens. The immune system detects these signals (microbial-associated molecular patterns) through receptors such as toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors; NLRs), and reacts through innate (antimicrobial peptides, protein of the mucus) and adaptive immunity (both cellular and humoral) responses. The result is a complex, highly multifactorial, and adaptive network of consequences which favor some bacterial species and disadvantages others. IST can interfere with basically all the components cited here and it is very complicated to prove that the decrease (or the increase) of one specific bacterial group is the direct consequence of one immunological pathway. In this paragraph, we provide succinct tracks to explore the topic.

9.1. The direct effect of IST on bacterial growth

Many ISTs are antimicrobial in nature. Tacrolimus was first developed as an antibacterial and also has a direct antifungal activity. Thus, tacrolimus may alter the microbial gut balance. Similarly, rapamycin is a macrolide derived from *Streptomyces hygroscopicus* that is also effective against fungi[140]. However, to our knowledge, the direct effect of IST on specific GM components has not been studied. This is because the culture of many gut bacteria species remains challenging. Furthermore, the *in vivo* antimicrobial activity of IST probably depends on pharmacological parameters that are difficult to explore, including the difficult-to-establish IST drug concentrations in the various components of the digestive tract: mucosa, mucus and intestinal lumen. Mucus-associated GM communities are probably not affected in the same way by IST than communities residing in the lumen. Recently, Jalili-Firoozinezhad *et al*. were

able to establish a complex human GM culture *in vitro*[141]. This will be a critical tool to explore the direct effect of IST (and other treatments) on the GM.

9.2. Indirect effects of ISTs on the GM through the modification of host's immunity

Controlling GM overgrowth and containing bacteria within the digestive lumen (compartmentalization) are essential to the health of the host. As an example, *Myd88*$^{-/-}$ mice develop to harbor commensal bacteria in their spleen[45]. Furthermore, to ensure an adequate compartmentalization, both innate and humoral immunities are necessary. Indeed, *Myd88*$^{-/-}$ and *Ticam*$^{-/-}$ double-deficient mice not only harbor commensal mice in their spleen, but also spontaneously develop high titers of anti-commensal serum IgGs[45]. The control of the immune system over the GM has been extensively reviewed by Willing *et al.*[142]. As various IST can alter the gut mucus[55,58], the global production of immunoglobulins, neutrophils (producing steroids and MMF) and T- and B-cell function, it is probable that ISTs modify the GM indirectly through immunomodulation. In favor of this hypothesis we have found that prednisolone or a combination of prednisolone, tacrolimus and MMF significantly reduces the production of ileal IL-22 resulting in a decreased expression of the gut C-type lectins Reg3γ and Reg3β, two anti-microbial peptides that directly affect the GM[56]. Lastly, we have also shown that an IST comprising with prednisolone, tacrolimus and MMF is responsible for the proliferation of both commensal and extra-intestinal pathogenic *E. coli*[56]. It is very unlikely that this bacterial proliferation is a direct consequence of IST on *E. coli*, rather than an indirect consequence through immunomodulation.

10. Limitations

10.1. Variability of studies on rodent GM.

The different studies on the impact of IS drugs on the GM show similarities but also differences, making it difficult to draw a reproducible and reliable phenotype of IS-induced gut dysbiosis.

Many factors contribute to this heterogeneity of results. Indeed, the GM is highly variable and many environmental factors can modulate its composition. Among these are chow composition, husbandry ventilation, barrier access, day/light cycle, and other animal facility parameters[143–145].

Some intrinsic factors may also explain inter-study microbiota variation such as rodent age or rodent strain[146,147]. Laboratory techniques for DNA extraction and microbiota analysis also influence results[148]. Some authors have suggested a quality control procedure to homogenize research on the GM, in order to increase its reproducibility[149]. It is also important to consider that IST-induced effects probably depend on the initial composition of the GM. We have shown that the effect of IST on the GM differed between two identically designed replicates of an experiment probably because of a different initial microbiota composition[56].

Finally, it is probable that the effects of IST on the GM differ according to the dose used in each study[97].

10.2. Extrinsic validity of rodent GM studies.

Husbandries are required to maintain strict hygienic rules to avoid sanitary disasters. As a consequence, mice raised in these aseptic environments exhibit differences in their gut, vaginal and skin microbiota, and in their immune systems compared to “dirty mammalians” [150]. For that reason, the generalization to wild mice is limited. Interventions such as the “wilding” of laboratory mice could decrease the physiologic and metagenomic gap between laboratory rodents and free-ranging mammals including humans[150].

11. Conclusions.

The GM is altered after SOT and these modifications, both before and after transplant are associated with post-transplantation adverse events. IST reshapes the GM and alter its structural and functional composition. Because IST-induced dysbiosis might contribute to these adverse events, a better understanding of the genesis of this dysbiosis is necessary.

On the one hand IST-induced dysbiosis probably participates in the immunosuppressive effects of these treatments and could improve allograft survival. On the other hand, alterations of the GM may be linked to IST-related adverse events such as metabolic disorders, colonic inflammation or intestinal colonization by pathogens.

ISTs deeply affect the intestinal barrier, from the mucus secretion to mucosal immunity[151] and intestinal permeability. Another level of complexity is that IST-induced gut barrier alterations have been shown to be both a cause and a consequence of GM modification. The depletion of the microbiota associated with the weakening of the intestinal barrier could promote the colonization of pathogenic bacteria observed in several studies. Finally, GM also modulates IS drug metabolism which probably participates to the inter-patient variability of IST dosing to reach therapeutic trough levels.

A better understanding of GM implication in SOT could thus help clinicians to prevent the occurrence of post-transplant adverse outcomes.

In the future, modulation of the intestinal microbiota, either by the use of probiotics or by the transfer of fecal microbiota could help prolong the survival of the graft and limit the occurrence of side effects related to immunosuppressive treatments.

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Figure legends

Figure 1: Factors which affect the intestinal microbiota after kidney transplantation. These can be divided in pharmacological factors, such as anti-infectious treatments[26], immunosuppressive drugs (this review) and anesthetics[152], and non-pharmacological factors, such as the normalization of renal function and associated metabolic abnormalities[124], the modification of dietary habits[153] and the discontinuation of chronic hemodialysis[154]

Figure 2: Impact of glucocorticoids (GCs) on the gut microbiota (GM). GCs alter the gut microbiota composition at different phylogenic levels depending on the studies, the dosing and the host (patients, or rodent model). Under physiological conditions, GCs inhibit the expression and synthesis of Muc2, the main component of colonic mucus. GCs also alter gut immunity: first, they downregulate the ileal expression of antimicrobial lectins RegIII β and Reg III ɣ, via the inhibition of IL-22; second, they restrict the coating of bacteria by mucosal IgA. On the other hand, GCs induce a retightening of TNF-α-induced tight junction relaxation by downregulating myosin light chain kinase (MLCK) synthesis and Myosin Light Chain 2 (MLC2) phosphorylation. These modifications of the gut barrier may cause GM modifications. The dysregulation of the circadian clock by exogenous GCs could also result in gut dysbiosis. In mice, GCs delay the restoration of the GM after antibiotic treatment, exposing the animals to more severe *Clostridium difficile* infection. Finally, *C. scindens* converts GCs into androgens, implicating the GM in the metabolism of GCs.
GCs: glucocorticoids. GM: gut microbiota. Muc2: mucin 2. MLC2: Myosin Light Chain 2. MLCK: MLC Kinase. Some elements of the Figure were obtained from Servier Medical Art®.

Figure 3: Impact of tacrolimus on the gut microbiota. Tacrolimus-induced dysbiosis results in functional alterations of the GM. Tacrolimus confers immunosuppressive properties to the GM both at the local and the systemic levels by increasing the population of T_{reg} lymphocytes. Tacrolimus-induced GM alterations could also result in some of the drug's side effects such as high blood pressure and diabetes. Tacrolimus increases the gut permeability

and decreases ileal RegIIIβ levels, participating in the dysbiosis. Tacrolimus is converted by *F. prausnitzii* or *Clostridiales* into a 15-fold less active compound called "M1".
GM: gut microbiota. T_{reg}: Regulatory T cells.
Some elements of the Figure were obtained from Servier Medical Art®.

Figure 4: Impact of mycophenolate mofetil (MMF) on the gut microbiota. MMF strips the diversity of the GM, increases the ratio Firmicutes/Bacteroidetes, and favors the Proteobacteria, a phylum that harbors pathogenic strains such as *Shigella* and *E. coli*. This gut dysbiosis generates high fecal concentrations of lipopolysaccharides (LPS) and colonic inflammation. In addition, mycophenolic acid (MPA), the active metabolite of MMF, perturbs tight junctions by upregulating Myosin Light Chain Kinase (MLCK) and Myosin Light Chain 2 (MLC2) phosphorylation. Combined, this is responsible for endotoxemia, a phenomenon that is associated with a higher rate of cardiovascular events after KT. Finally, the abundance of *Bacteroides sp.* correlates with a high level of activity of colonic bacterial β-glucuronidase, an enzyme that converts the glucuronated form of MPA (MPAG) back into its active form. Modulation of the GM with antibiotics reduces β-glucuronidase activity, decreases colonic MPA levels and amends MMF's digestive side effects.
MMF: mycophenolate mofetil. GM: gut microbiota. LPS: lipopolysaccharides. KT: kidney transplant. MPA: mycophenolic acid. MLC2: Myosin Light Chain 2. MLCK: MLC Kinase.
Some elements of the Figure were obtained from Servier Medical Art®.

Figure 5: Impact of combined therapy on the gut microbiota. A combination of tacrolimus, MMF and prednisolone promotes both commensal and extra-intestinal pathogenic *E. coli* proliferation and decreases the ileal relative abundance of *Clostridium sensu stricto.* Combined therapy decreases ileal gut immunity by decreasing IL-22, RegIIIβ and RegIIIγ expression.
MMF: mycophenolate mofetil.
Some elements of the Figure were obtained from Servier Medical Art®.

Figure 6: Symbols used in the Figures

References

1. Thursby, E. & Juge, N. Introduction to the human gut microbiota. *Biochem. J.* **474**, 1823–1836 (2017).
2. Savage, D. C. Microbial Ecology of the Gastrointestinal Tract. *Annu. Rev. Microbiol.* **31**, 107–133 (1977).
3. Lynch, S. V. & Pedersen, O. The Human Intestinal Microbiome in Health and Disease. *N. Engl. J. Med.* **375**, 2369–2379 (2016).
4. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
5. Kamada, N., Chen, G. Y., Inohara, N. & Núñez, G. Control of Pathogens and Pathobionts by the Gut Microbiota. *Nat. Immunol.* **14**, 685–690 (2013).
6. Hill, D. A. & Artis, D. Intestinal Bacteria and the Regulation of Immune Cell Homeostasis. *Annu. Rev. Immunol.* **28**, 623–667 (2010).
7. Backhed, F. *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci.* **101**, 15718–15723 (2004).
8. Fung, T. C., Olson, C. A. & Hsiao, E. Y. Interactions between the microbiota, immune and nervous systems in health and disease. *Nat. Neurosci.* **20**, 145–155 (2017).
9. Heijtz, R. D. *et al.* Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 3047–3052 (2011).
10. Claus, S. P. *et al.* Colonization-Induced Host-Gut Microbial Metabolic Interaction. *mBio* **2**, (2011).
11. Vanlancker, E., Vanhoecke, B., Stringer, A. & de Wiele, T. V. 5-Fluorouracil and irinotecan (SN-38) have limited impact on colon microbial functionality and composition in vitro. 20 (2017).

12. Li, J. *et al.* Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* **5**, (2017).

13. Oyama, J. & Node, K. Gut microbiota and hypertension. *Hypertens. Res.* **42**, 741–743 (2019).

14. Yang, T. *et al.* Gut Dysbiosis Is Linked to Hypertension. *Hypertension* **65**, 1331–1340 (2015).

15. Honour, J. W. Historical perspective: gut dysbiosis and hypertension. *Physiol. Genomics* **47**, 443–446 (2015).

16. the IMI-DIRECT consortium *et al.* Aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia* **61**, 810–820 (2018).

17. Tilg, H. & Moschen, A. R. Microbiota and diabetes: an evolving relationship. *Gut* **63**, 1513–1521 (2014).

18. Larsen, N. *et al.* Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. *PLoS ONE* **5**, e9085 (2010).

19. Cani, P. D. *et al.* Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. *Diabetes* **56**, 1761–1772 (2007).

20. Parashar, A. & Udayabanu, M. Gut microbiota: Implications in Parkinson's disease. *Parkinsonism Relat. Disord.* **38**, 1–7 (2017).

21. Hu, X., Wang, T. & Jin, F. Alzheimer's disease and gut microbiota. *Sci. China Life Sci.* **59**, 1006–1023 (2016).

22. Sokol, H. *et al.* Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm. Bowel Dis.* **12**, 106–111 (2006).

23. Mazmanian, S. K., Round, J. L. & Kasper, D. L. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* **453**, 620–625 (2008).

24. Barnich, N. & Darfeuille-Michaud, A. Role of bacteria in the etiopathogenesis of inflammatory bowel disease. *World J. Gastroenterol. WJG* **13**, 5571–5576 (2007).

25. Maurice, C. F., Haiser, H. J. & Turnbaugh, P. J. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* **152**, 39–50 (2013).

26. Modi, S. R., Collins, J. J. & Relman, D. A. Antibiotics and the gut microbiota. *J. Clin. Invest.* **124**, 4212–4218 (2014).

27. Pérez-Cobas, A. E. *et al.* Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut* **62**, 1591–1601 (2013).

28. Zwielehner, J. *et al.* Changes in Human Fecal Microbiota Due to Chemotherapy Analyzed by TaqMan-PCR, 454 Sequencing and PCR-DGGE Fingerprinting. *PLoS ONE* **6**, e28654 (2011).

29. Montassier, E. *et al.* Chemotherapy-driven dysbiosis in the intestinal microbiome. *Aliment. Pharmacol. Ther.* **42**, 515–528 (2015).

30. Montassier, E. *et al.* 16S rRNA Gene Pyrosequencing Reveals Shift in Patient Faecal Microbiota During High-Dose Chemotherapy as Conditioning Regimen for Bone Marrow Transplantation. *Microb. Ecol.* **67**, 690–699 (2014).

31. Le Bastard, Q. *et al.* Fecal microbiota transplantation reverses antibiotic and chemotherapy-induced gut dysbiosis in mice. *Sci. Rep.* **8**, 6219 (2018).

32. Million, M. *et al.* Lactobacillus reuteri and Escherichia coli in the human gut microbiota may predict weight gain associated with vancomycin treatment. *Nutr. Diabetes* **3**, e87 (2013).

33. Wu, H. *et al.* Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat. Med.* **23**, 850–858 (2017).

34. Vetizou, M. *et al.* Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079–1084 (2015).

35. The global observatory on donation and transplantation. *http://www.transplant-observatory.org* (2019).

36. Zaza, G., Granata, S., Tomei, P., Dalla Gassa, A. & Lupo, A. Personalization of the Immunosuppressive Treatment in Renal Transplant Recipients: The Great Challenge in “Omics” Medicine. *Int. J. Mol. Sci.* **16**, 4281–4305 (2015).

37. Round, J. L. & Mazmanian, S. K. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**, 313–323 (2009).

38. Levy, M., Kolodziejczyk, A. A., Thaiss, C. A. & Elinav, E. Dysbiosis and the immune system. *Nat. Rev. Immunol.* **17**, 219–232 (2017).

39. Sommer, F. & Backhed, F. The gut microbiota--masters of host development and physiology. *Nat. Rev. Microbiol.* **11**, 227–238 (2013).

40. Knauf, F., Brewer, J. R. & Flavell, R. A. Immunity, microbiota and kidney disease. *Nat. Rev. Nephrol.* **15**, 263–274 (2019).

41. Round, J. L. *et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* **332**, 974–977 (2011).

42. Anders, H.-J., Andersen, K. & Stecher, B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int.* **83**, 1010–1016 (2013).

43. Vaziri, N. D. *et al.* Chronic kidney disease alters intestinal microbial flora. *Kidney Int.* **83**, 308–315 (2013).

44. Brandl, K. *et al.* Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. *Nature* **455**, 804–807 (2008).

45. Slack, E. *et al.* Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620 (2009).

46. Wu, Z.-W. *et al.* Changes of gut bacteria and immune parameters in liver transplant recipients. *Hepatobiliary Pancreat. Dis. Int.* **11**, 40–50 (2012).

47. Kim, S. I. Bacterial infection after liver transplantation. *World J. Gastroenterol. WJG* **20**, 6211–6220 (2014).

48. Fricke, W. F., Maddox, C., Song, Y. & Bromberg, J. S. Human Microbiota Characterization in the Course of Renal Transplantation: Microbiota Changes During Renal Transplantation. *Am. J. Transplant.* **14**, 416–427 (2014).

49. Lee, J. R. *et al.* Gut Microbial Community Structure and Complications Following Kidney Transplantation: A Pilot Study. *Transplantation* **98**, 697–705 (2014).

50. Shin, N.-R., Whon, T. W. & Bae, J.-W. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* **33**, 496–503 (2015).

51. Lecronier, M. *et al.* Gut microbiota composition alterations are associated with the onset of diabetes in kidney transplant recipients. *PloS One* **15**, e0227373 (2020).

52. Lee, J. R. *et al.* Gut Microbial Community Structure and Complications After Kidney Transplantation: A Pilot Study. *Transplantation* **98**, 697–705 (2014).

53. Koh, A., De Vadder, F., Kovatcheva-Datchary, P. & Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **165**, 1332–1345 (2016).

54. Steiner, R. W. & Awdishu, L. Steroids in kidney transplant patients. *Semin. Immunopathol.* **33**, 157–167 (2011).

55. Wu, T. *et al.* Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats. *Life Sci.* **192**, 173–182 (2018).

56. Tourret, J. *et al.* Immunosuppressive Treatment Alters Secretion of Ileal Antimicrobial Peptides and Gut Microbiota, and Favors Subsequent Colonization by Uropathogenic Escherichia coli: *Transplantation* **101**, 74–82 (2017).

57. He, Z., Kong, X., Shao, T., Zhang, Y. & Wen, C. Alterations of the Gut Microbiota Associated With Promoting Efficacy of Prednisone by Bromofuranone in MRL/lpr Mice. *Front. Microbiol.* **10**, 978 (2019).

58. Huang, E. Y. *et al.* Using corticosteroids to reshape the gut microbiome: implications for inflammatory bowel diseases. *Inflamm. Bowel Dis.* **21**, 963–972 (2015).

59. Rodríguez-Piñeiro, A. M. & Johansson, M. E. V. The colonic mucus protection depends on the microbiota. *Gut Microbes* **6**, 326–330 (2015).

60. Bunker, J. J. *et al.* Innate and adaptive humoral responses coat distinct commensal bacteria with immunoglobulin A. *Immunity* **43**, 541–553 (2015).

61. Sommer, F., Anderson, J. M., Bharti, R., Raes, J. & Rosenstiel, P. The resilience of the intestinal microbiota influences health and disease. *Nat. Rev. Microbiol.* **15**, 630–638 (2017).

62. Kim, H. B., Wang, Y. & Sun, X. A Detrimental Role of Immunosuppressive Drug, Dexamethasone, During Clostridium difficile Infection in Association with a Gastrointestinal Microbial Shift. *J. Microbiol. Biotechnol.* **26**, 567–571 (2016).

63. Edgar, R. S. *et al.* Peroxiredoxins are conserved markers of circadian rhythms. *Nature* **485**, 459–464 (2012).

64. Thaiss, C. A. *et al.* Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostasis. *Cell* **159**, 514–529 (2014).

65. So, A. Y.-L., Bernal, T. U., Pillsbury, M. L., Yamamoto, K. R. & Feldman, B. J. Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 17582–17587 (2009).

66. Bordag, N. *et al.* Glucocorticoid (dexamethasone)-induced metabolome changes in healthy males suggest prediction of response and side effects. *Sci. Rep.* **5**, (2015).

67. Desai, M. S. *et al.* A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell* **167**, 1339-1353.e21 (2016).

68. Sicard, J.-F., Le Bihan, G., Vogeleer, P., Jacques, M. & Harel, J. Interactions of Intestinal Bacteria with Components of the Intestinal Mucus. *Front. Cell. Infect. Microbiol.* **7**, (2017).

69. Chelakkot, C., Ghim, J. & Ryu, S. H. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp. Mol. Med.* **50**, 103 (2018).

70. Schroeder, B. O. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. *Gastroenterol. Rep.* **7**, 3–12 (2019).

71. Muniz, L. R., Knosp, C. & Yeretssian, G. Intestinal antimicrobial peptides during homeostasis, infection, and disease. *Front. Immunol.* **3**, (2012).

72. Pabst, O. New concepts in the generation and functions of IgA. *Nat. Rev. Immunol.* **12**, 821–832 (2012).

73. Alverdy, J. & Aoys, E. The effect of glucocorticoid administration on bacterial translocation. Evidence for an acquired mucosal immunodeficient state. *Ann. Surg.* **214**, 719–723 (1991).

74. Palm, N. W. *et al.* Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* **158**, 1000–1010 (2014).

75. Boivin, M. A. *et al.* Mechanism of glucocorticoid regulation of the intestinal tight junction barrier. *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G590–G598 (2007).

76. Ridlon, J. M. *et al. Clostridium scindens* : a human gut microbe with a high potential to convert glucocorticoids into androgens. *J. Lipid Res.* **54**, 2437–2449 (2013).

77. Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R. & Goodman, A. L. Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature* **570**, 462–467 (2019).

78. Anaerobic and aerobic cleavage of the steroid core ring structure by Steroidobacter denitrificans - PubMed. https://pubmed.ncbi.nlm.nih.gov/23458847/.

79. Zhang, H., Ji, Y., Wang, Y., Zhang, X. & Yu, Y. Cloning and characterization of a novel β-ketoacyl-ACP reductase from Comamonas testosteroni. *Chem. Biol. Interact.* **234**, 213–220 (2015).

80. Ly, L. K. *et al.* Bacterial steroid-17,20-desmolase is a taxonomically rare enzymatic pathway that converts prednisone to 1,4-androstanediene-3,11,17-trione, a metabolite that causes proliferation of prostate cancer cells. *J. Steroid Biochem. Mol. Biol.* **199**, 105567 (2020).

81. So, S. Y. & Savidge, T. C. Sex-bias in irritable bowel syndrome: linking steroids to the gut-brain axis. *Front. Endocrinol.* **12**, 574 (2021).

82. Thomson, A. W., Bonham, C. A. & Zeevi, A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther. Drug Monit.* **17**, 584–591 (1995).

83. Ekberg, H., Vítko, Š., Margreiter, R., Frei, U. & Halloran, P. F. Reduced Exposure to Calcineurin Inhibitors in Renal Transplantation. *N Engl J Med* 14 (2007).

84. Heisel, O., Heisel, R., Balshaw, R. & Keown, P. New Onset Diabetes Mellitus in Patients Receiving Calcineurin Inhibitors: A Systematic Review and Meta-Analysis. *Am. J. Transplant.* **4**, 583–595 (2004).

85. Hoorn, E. J. *et al.* Pathogenesis of calcineurin inhibitor–induced hypertension. *J. Nephrol.* **25**, 269–275 (2012).

86. Bhat, M. *et al.* Impact of Immunosuppression on the Metagenomic Composition of the Intestinal Microbiome: a Systems Biology Approach to Post-Transplant Diabetes. *Sci. Rep.* **7**, 10277 (2017).

87. Zhang, Z. *et al.* Immunosuppressive effect of the gut microbiome altered by high-dose tacrolimus in mice. *Am. J. Transplant.* **18**, 1646–1656 (2018).

88. Toral, M. *et al. Lactobacillus fermentum* Improves Tacrolimus-Induced Hypertension by Restoring Vascular Redox State and Improving eNOS Coupling. *Mol. Nutr. Food Res.* **62**, 1800033 (2018).

89. Dimitriu, P. A. *et al.* Temporal stability of the mouse gut microbiota in relation to innate and adaptive immunity. *Environ. Microbiol. Rep.* **5**, 200–210 (2013).

90. Turnbaugh, P. J. *et al.* A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).

91. Ley, R. E. *et al.* Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 11070–11075 (2005).

92. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).

93. Pluznick, J. L. Microbial Short-Chain Fatty Acids and Blood Pressure Regulation. *Curr. Hypertens. Rep.* **19**, 25 (2017).

94. Sanna, S. *et al.* Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat. Genet.* **51**, 600–605 (2019).

95. Qin, J. *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).

96. Konikoff, T. & Gophna, U. Oscillospira: a Central, Enigmatic Component of the Human Gut Microbiota. *Trends Microbiol.* **24**, 523–524 (2016).

97. Jiang, J.-W. *et al.* Optimal immunosuppressor induces stable gut microbiota after liver transplantation. *World J. Gastroenterol.* **24**, 3871–3883 (2018).

98. Langille, M. G. I. *et al.* Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **31**, 814–821 (2013).

99. van Dieren, J. M. *et al.* Local Immune Regulation of Mucosal Inflammation by Tacrolimus. *Dig. Dis. Sci.* **55**, 2514–2519 (2010).

100. Lee, J. R. *et al.* Gut Microbiota and Tacrolimus Dosing in Kidney Transplantation. *PLOS ONE* **10**, e0122399 (2015).

101. Bekersky, I., Dressler, D. & Mekki, Q. A. Effect of Low- and High-Fat Meals on Tacrolimus Absorption following 5 mg Single Oral Doses to Healthy Human Subjects. *J. Clin. Pharmacol.* **41**, 176–182 (2001).

102. Chen, L. & Prasad, G. V. R. CYP3A5 polymorphisms in renal transplant recipients: influence on tacrolimus treatment. *Pharmacogenomics Pers. Med.* **Volume 11**, 23–33 (2018).

103. Guo, Y. *et al.* Commensal Gut Bacteria Convert the Immunosuppressant Tacrolimus to Less Potent Metabolites. *Drug Metab. Dispos.* **47**, 194–202 (2019).

104. Freeman, D. J. Pharmacology and pharmacokinetics of cyclosporine. *Clin. Biochem.* **24**, 9–14 (1991).

105. Jia, J. *et al.* Structural shifts in the intestinal microbiota of rats treated with cyclosporine A after orthotropic liver transplantation. *Front. Med.* **13**, 451–460 (2019).

106. Lamba, V. *et al.* PharmGKB summary: mycophenolic acid pathway. *Pharmacogenet. Genomics* **24**, 73–79 (2014).

107. Behrend, M. Adverse Gastrointestinal Effects of Mycophenolate Mofetil: Aetiology, Incidence and Management. *Drug Saf.* **24**, 645–663 (2001).

108. Calmet, F. H., Yarur, A. J., Pukazhendhi, G., Ahmad, J. & Bhamidimarri, K. R. Endoscopic and histological features of mycophenolate mofetil colitis in patients after solid organ transplantation. *Ann. Gastroenterol.* 8.

109. Bunnapradist, S. *et al.* Mycophenolate Mofetil Dose Reductions and Discontinuations after Gastrointestinal Complications Are Associated with Renal Transplant Graft Failure: *Transplantation* **82**, 102–107 (2006).

110. Taylor, M. R. *et al.* Vancomycin relieves mycophenolate mofetil–induced gastrointestinal toxicity by eliminating gut bacterial β-glucuronidase activity. *Sci. Adv.* **5**, eaax2358 (2019).

111. Flannigan, K. L. *et al.* An intact microbiota is required for the gastrointestinal toxicity of the immunosuppressant mycophenolate mofetil. *J. Heart Lung Transplant.* **37**, 1047–1059 (2018).

112. Cani, P. D. *et al.* Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. *Diabetes* **56**, 1761–1772 (2007).

113. Chan, W. *et al.* The Associations of Endotoxemia With Systemic Inflammation, Endothelial Activation, and Cardiovascular Outcome in Kidney Transplantation. *J. Ren. Nutr.* **28**, 13–27 (2018).

114. Qasim, M., Rahman, H., Ahmed, R., Oellerich, M. & Asif, A. R. Mycophenolic acid mediated disruption of the intestinal epithelial tight junctions. *Exp. Cell Res.* **322**, 277–289 (2014).

115. Prolonged Survival of Human-Kidney Homografts by Immunosuppressive Drug Therapy | NEJM. https://www.nejm.org/doi/full/10.1056/NEJM196306132682401.

116. Maltzman, J. S. & Koretzky, G. A. Azathioprine: old drug, new actions. *J. Clin. Invest.* **111**, 4 (2003).

117. Anstey, A. & Lear, J. T. Azathioprine: Clinical Pharmacology and Current Indications in Autoimmune Disorders. *BioDrugs* **9**, 33–47 (1998).

118. Liu, F. *et al.* Azathioprine, Mercaptopurine, and 5-Aminosalicylic Acid Affect the Growth of IBD-Associated Campylobacter Species and Other Enteric Microbes. *Front. Microbiol.* **8**, (2017).

119. Swidsinski, A., Loening-Baucke, V., Bengmark, S., Lochs, H. & Dörffel, Y. Azathioprine and mesalazine-induced effects on the mucosal flora in patients with IBD colitis: *Inflamm. Bowel Dis.* **13**, 51–56 (2007).

120. Sehgal, S. N. Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant. Proc.* **35**, S7–S14 (2003).

121. Ventura-Aguiar, P., Campistol, J. M. & Diekmann, F. Safety of mTOR inhibitors in adult solid organ transplantation. *Expert Opin. Drug Saf.* **15**, 303–319 (2016).

122. Jung, M.-J. *et al.* Chronic Repression of mTOR Complex 2 Induces Changes in the Gut Microbiota of Diet-induced Obese Mice. *Sci. Rep.* **6**, 30887 (2016).

123. Schinaman, J. M., Rana, A., Ja, W. W., Clark, R. I. & Walker, D. W. Rapamycin modulates tissue aging and lifespan independently of the gut microbiota in Drosophila. *Sci. Rep.* **9**, 7824 (2019).

124. Sampaio-Maia, B., Simões-Silva, L., Pestana, M., Araujo, R. & Soares-Silva, I. J. The Role of the Gut Microbiome on Chronic Kidney Disease. in *Advances in Applied Microbiology* vol. 96 65–94 (Elsevier, 2016).

125. Zhou, Y., Rychahou, P., Wang, Q., Weiss, H. L. & Evers, B. M. TSC2/mTORC1 signaling controls Paneth and goblet cell differentiation in the intestinal epithelium. *Cell Death Dis.* **6**, e1631 (2015).

126. Faller, W. J. *et al.* mTORC1 mediated translational elongation limits intestinal tumour initiation and growth. *Nature* **517**, 497–500 (2015).

127. Xie, Y. *et al.* Gut epithelial TSC1/mTOR controls RIPK3-dependent necroptosis in intestinal inflammation and cancer. https://www.jci.org/articles/view/133264/pdf (2020) doi:10.1172/JCI133264.

128. Hurez, V. *et al.* Chronic mTOR inhibition in mice with rapamycin alters T, B, myeloid, and innate lymphoid cells and gut flora and prolongs life of immune-deficient mice. *Aging Cell* **14**, 945–956 (2015).

129. Vincenti, F. *et al.* Belatacept and Long-Term Outcomes in Kidney Transplantation. *N. Engl. J. Med.* **374**, 333–343 (2016).

130. Larsen, C. P. *et al.* Rational Development of LEA29Y (belatacept), a High-Affinity Variant of CTLA4-Ig with Potent Immunosuppressive Properties. *Am. J. Transplant.* **5**, 443–453 (2005).

131. Bozon, A. *et al.* Stricturing Crohn's disease-like colitis in a patient treated with belatacept. *World J. Gastroenterol.* **23**, 8660–8665 (2017).

132. Flynn, J. M. & Byrd, J. C. Campath-1H monoclonal antibody therapy: *Curr. Opin. Oncol.* **12**, 574–581 (2000).

133. Hanaway, M. J., Peddi, V. R. & Croy, R. Alemtuzumab Induction in Renal Transplantation. *N. Engl. J. Med.* 11 (2011).

134. Li, Q. R. *et al.* Reciprocal Interaction Between Intestinal Microbiota and Mucosal Lymphocyte in Cynomolgus Monkeys After Alemtuzumab Treatment: Intestinal Microbiota and Mucosal Lymphocyte. *Am. J. Transplant.* **13**, 899–910 (2013).

135. Li, Q., Wang, C., Tang, C., He, Q. & Li, J. Lymphocyte Depletion After Alemtuzumab Induction Disrupts Intestinal Fungal Microbiota in Cynomolgus Monkeys. **98**, 9 (2014).

136. Jones-Hughes, T. *et al.* Immunosuppressive therapy for kidney transplantation in adults: a systematic review and economic model. *Health Technol. Assess. Winch. Engl.* **20**, 1–594 (2016).

137. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* **9 Suppl 3**, S1-155 (2009).

138. Pellé, G. *et al.* Acute Pyelonephritis Represents a Risk Factor Impairing Long-Term Kidney Graft Function. *Am. J. Transplant.* **7**, 899–907 (2007).

139. Zaza, G. *et al.* Impact of maintenance immunosuppressive therapy on the fecal microbiome of renal transplant recipients: Comparison between an everolimus- and a standard tacrolimus-based regimen. *PLOS ONE* **12**, e0178228 (2017).

140. Husain, S. & Singh, N. The Impact of Novel Immunosuppressive Agents on Infections in Organ Transplant Recipients and the Interactions of These Agents with Antimicrobials. *Clin. Infect. Dis.* **35**, 53–61 (2002).

141. Jalili-Firoozinezhad, S. *et al.* A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat. Biomed. Eng.* **3**, 520–531 (2019).

142. Willing, B. P., Gill, N. & Finlay, B. B. The role of the immune system in regulating the microbiota. *Gut Microbes* **1**, 213–223 (2010).

143. Laukens, D., Brinkman, B. M., Raes, J., De Vos, M. & Vandenabeele, P. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. *FEMS Microbiol. Rev.* **40**, 117–132 (2016).

144. Rausch, P. *et al.* Analysis of factors contributing to variation in the C57BL/6J fecal microbiota across German animal facilities. *Int. J. Med. Microbiol.* **306**, 343–355 (2016).

145. Parker, K. D., Albeke, S. E., Gigley, J. P., Goldstein, A. M. & Ward, N. L. Microbiome Composition in Both Wild-Type and Disease Model Mice Is Heavily Influenced by Mouse Facility. *Front. Microbiol.* **9**, 1598 (2018).

146. Campbell, J. H. *et al.* Host genetic and environmental effects on mouse intestinal microbiota. *ISME J.* **6**, 2033–2044 (2012).

147. Flemer, B. *et al.* Fecal microbiota variation across the lifespan of the healthy laboratory rat. *Gut Microbes* **8**, 428–439 (2017).

148. Thomas, V., Clark, J. & Dore, J. Fecal microbiota analysis: an overview of sample collection methods and sequencing strategies. *Future Microbiol.* **10**, 1485–1504 (2015).

149. Stappenbeck, T. S. & Virgin, H. W. Accounting for reciprocal host–microbiome interactions in experimental science. *Nature* **534**, 191–199 (2016).

150. Rosshart, S. P. *et al.* Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science* **365**, eaaw4361 (2019).

151. Sonnenberg, G. F. & Artis, D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. *Immunity* **37**, 601–610 (2012).

152. Serbanescu, M. A. *et al.* General Anesthesia Alters the Diversity and Composition of the Intestinal Microbiota in Mice. *Anesth. Analg.* **129**, e126–e129 (2019).

153. Conlon, M. A. & Bird, A. R. The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. *Nutrients* **7**, 17–44 (2014).

154. Jazani, N. H., Savoj, J., Lustgarten, M., Lau, W. L. & Vaziri, N. D. Impact of Gut Dysbiosis on Neurohormonal Pathways in Chronic Kidney Disease. *Diseases* **7**, (2019).

Table 1 : Overview of the effects of immunosuppressive therapy on the gut microbiota, and their consequences

Molecule	Microbial effects on the gut microbiota	Hypothetical clinical relevance	References
Tacrolimus	**Intra-peritoneal injections in rats:** Decreased: Shannon index Increased: Firmicutes/Bacteroidetes ratio	Increased Allobaculum as a gut signature of tacrolimus-induced immunosuppression Analogous dysbiosis as in metabolic diseases Depleting effect on short chain fatty acid producing bacteria which induces a dysregulation of blood pressure, lipogenesis, and blood glucose control. Decreased expression of occludin and Muc3 which increases intestinal permeability leading to endotoxemia	88
	Intra-peritoneal injections in rats: Decreased: Fecal Mollicutes, Micrococcaceae, Actinomycetales, *Roseburia, Oscillospira, Rothia, Staphylococcus* Increased: Fecal *A. muciniphila*		86,88,97
	Oral administration in mice: Decreased: Ruminococcaceae, *Clostridium*, *Rikenella*,	Gut signature of tacrolimus-induced immunosuppression	56,87,88

	Bifidobacterium Increased: *Allobaculum*, *Bacteroides*, *Lactobacillus*, *A. muciniphila* Decreasing RegIIIɣ expression leading to modifying of the bacterial density	High blood pressure Impaired lipids metabolism Impaired blood glucose regulation Depleting effect on short chain fatty acid production	
	Dose-dependent effect in a rat liver transplant model: **MIDDLE dose (0.5 mg/kg)** Decreased: Enterobacteriaceae, *Bacteroides-Prevotella* Increased: Microbial richness, *Bifidobacterium, Fecalibacterium prausnitzii*	**MIDDLE DOSE** Better liver transplant outcome	97

	LOW (0.1 mg/kg) or HIGH (1 mg/kg) doses Decreased: Microbial richness, *Bifidobacterium, Fecalibacterium prausnitzii* Increased: *Enterobacteriaceae*	**LOW or HIGH DOSES** Poorer allograft outcome and endotoxemia	
	Increased: *A. muciniphila* Decreased: *Roseburia*	Similar gut microbiota as type 2 diabetes (except for *A. muciniphila*)	[95]
	Tacrolimus treatment or fecal microbiota transfer: Increased proportion of $CD4^+$ $CD25^{hi}$ $FoxP3^+$ regulatory T cells (in the colonic mucosa and the circulation)	Effect on regulatory T cells mediated through the microbiota	[87]

Cyclosporine	**Rat model of allogenic liver transplantation:** Increased: Gut microbial richness, *F. prausnitzii* Decreased: *Enterobacteriaceae, Clostridium clusters I and XIV*	Identification of a "rejecting gut microbiota"	105
Corticosteroids	**Mouse model:** Decreased: Bacterial richness and diversity Alteration of the global composition of the GM Firmicutes, Bacteroidetes, Actinobacteria, alpha and gamma proteobacteria, *Deferribacteres, Oscillospira, Bilophila, Rikenella, Mucispillirum* *Clostridium spp.* in the ileum Increased: Firmicutes/Bacteroidetes ratio *Lactobacillus*, Clostridiales, Anaerostipes,	Disruption of circadian circle Increased delay of resilience leading to more severe *Clostridium difficile* infection Impaired activation and maturation of T cells	55–58,100,101

	Bifidobacterium		
	Decreased Muc2 expression and synthesis, diminution of C-type Lectin RegIIIɤ and RegIIIβ*:* Decrease level of IgA in the bile*:* Decrease TNF alpha level*:*	Impairment of immune response Decreased IgA bacterial coating and pathogens destruction	56,73,75
mTORi	**Rapamycin in rats and mice** <u>Decreased:</u> Phylogenetic diversity score, *Turicibacter,* unclassified Marinilabiliaceae*, Alloprevotella*, *unclassified Porphyromonadaceae, Ruminococcus, Bifidobacterium, Marvinbryantia, Helicobacter, Coprobacillus* <u>Increased:</u> *Ruminococcus* (Lachnospiraceae)	Pro-inflammatory cytokines release from Peyer's patches Metabolic disorders: body weight gain, insulin resistance, intestinal inflammation and fat deposit	86,122
	Rapamycin in rats		86

	Decreased: *Roseburia, Oscillospira,* Mollicutes, Rothia, Micrococcaceae, Actinomycetales, *Staphylococcus*		
	Everolimus in mice*:* Decreased ileal expression of RegIIIβ and RegIIIγ levels	Modification of innate immunity	56
MMF	**Mice model:** Decrease: Microbial richness, *Akkermansia*, Parabacteroides, *Clostridium* Increase: Firmicutes/Bacteroidetes ratio, Proteobacteria (including *Escherichia* and *Shigella), Clostridia, Bacteroides spp,* Clostridia, *Bacteroides spp*, activity of gut β-glucuronidase	Endotoxemia leading to low grade inflammation, promoting metabolic syndrome, insulin resistance and cardiovascular event Colonic atrophy, cecal atrophy and weight loss (Cellcept-induced colitis)	110,111

Azathioprine	Inhibited in vitro growth: *Campylobacter concisus*, *Bacteroides fragilis*, *Bacteroides vulgatus*, *E. coli* (only high concentrations)		118
	Human with IBD vs healthy controls: Increase: concentration of mucosal bacteria		119
Belatacept	Not determined	One case of Crohn -like colitis	131
Alemtuzumab	**Monkey model**: Increase: *Prevotella, E. coli, Shigella flexneri,* Clostridiales Decrease: *Fecalibacterium Prausnitzii*		134
Combined therapy	**In rodents**: Decrease: *Clostridium sensu stricto*	High prevalence of urinary tract infection	56

(Prednisolone, tacrolimus and MMF)	Increase: *E. coli*		

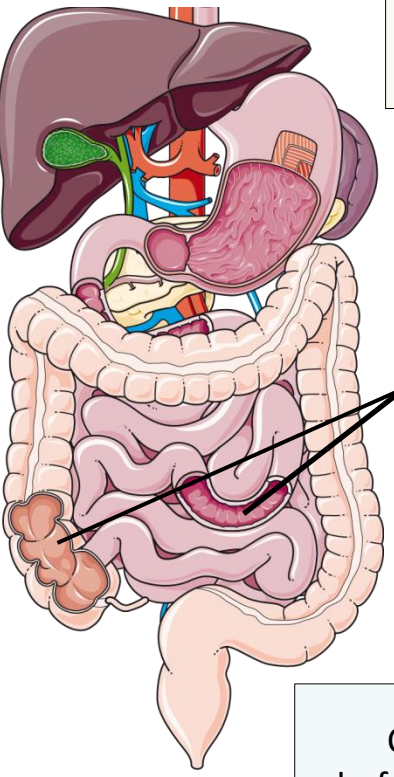
Pharmacological interventions

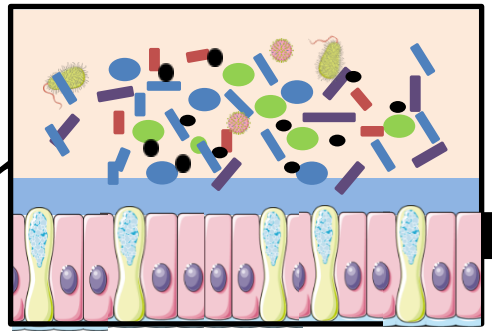
Immunosuppressive drugs

General anesthesia

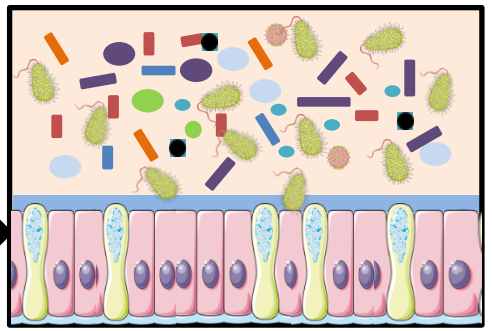
Anti-infectious treatments and prophylaxis

Discontinuation of drugs that are specific for end-stage organ failure





Kidney transplant



Gut microbiota modification

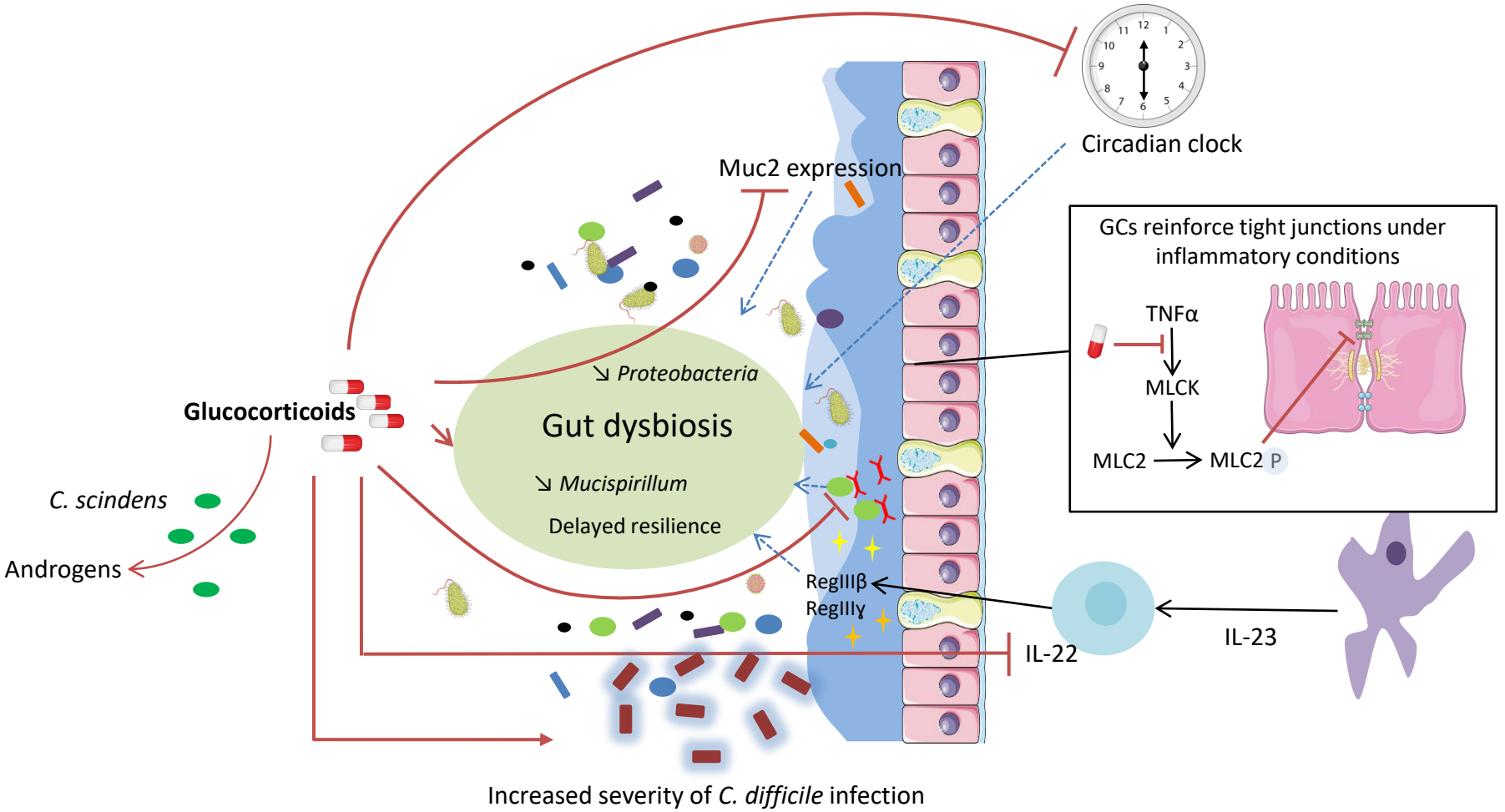
Correction of renal dysfunction (normalization of acidemia, uremia, hyperphostphatemia…)

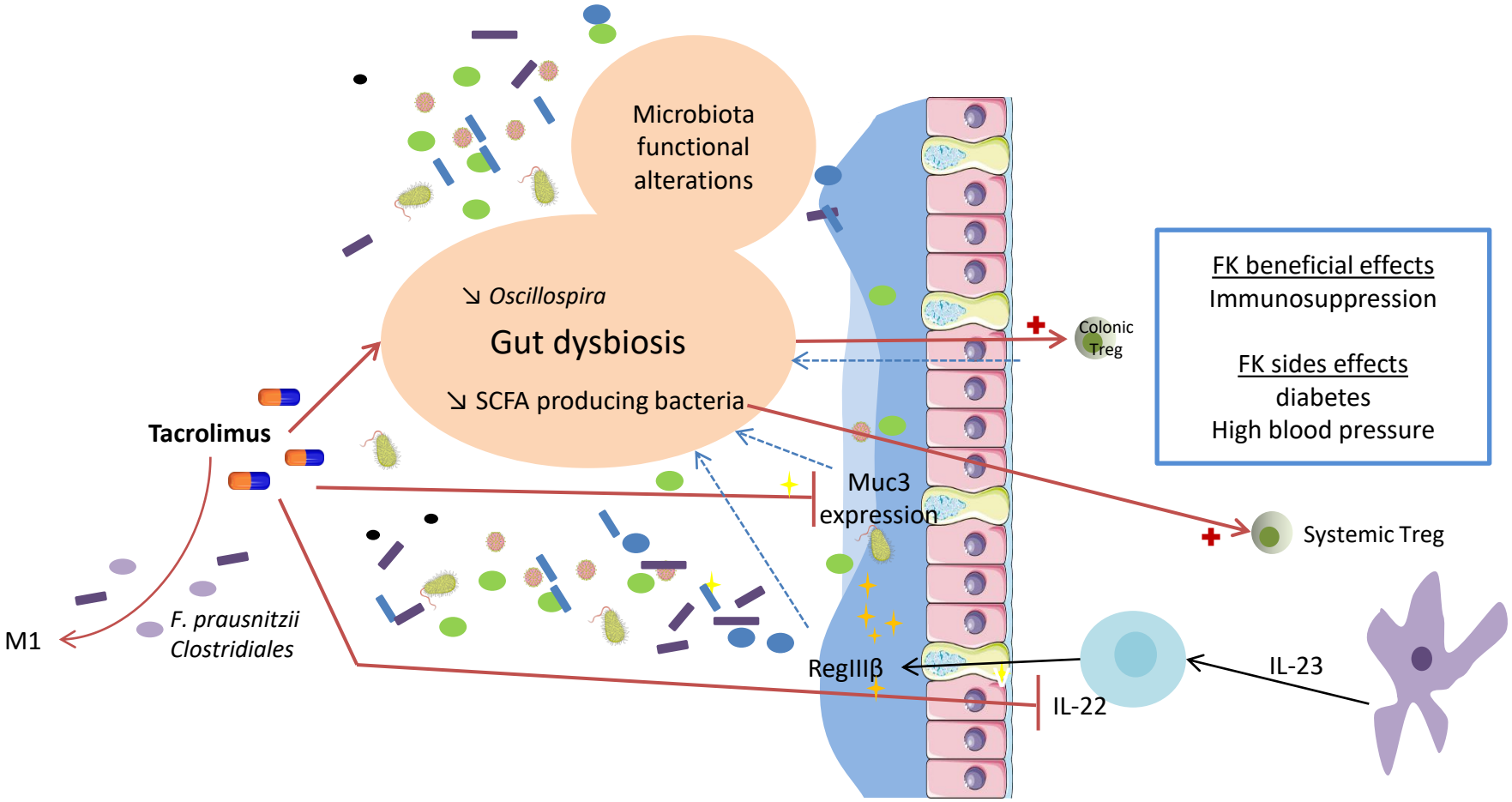
Dietary changes

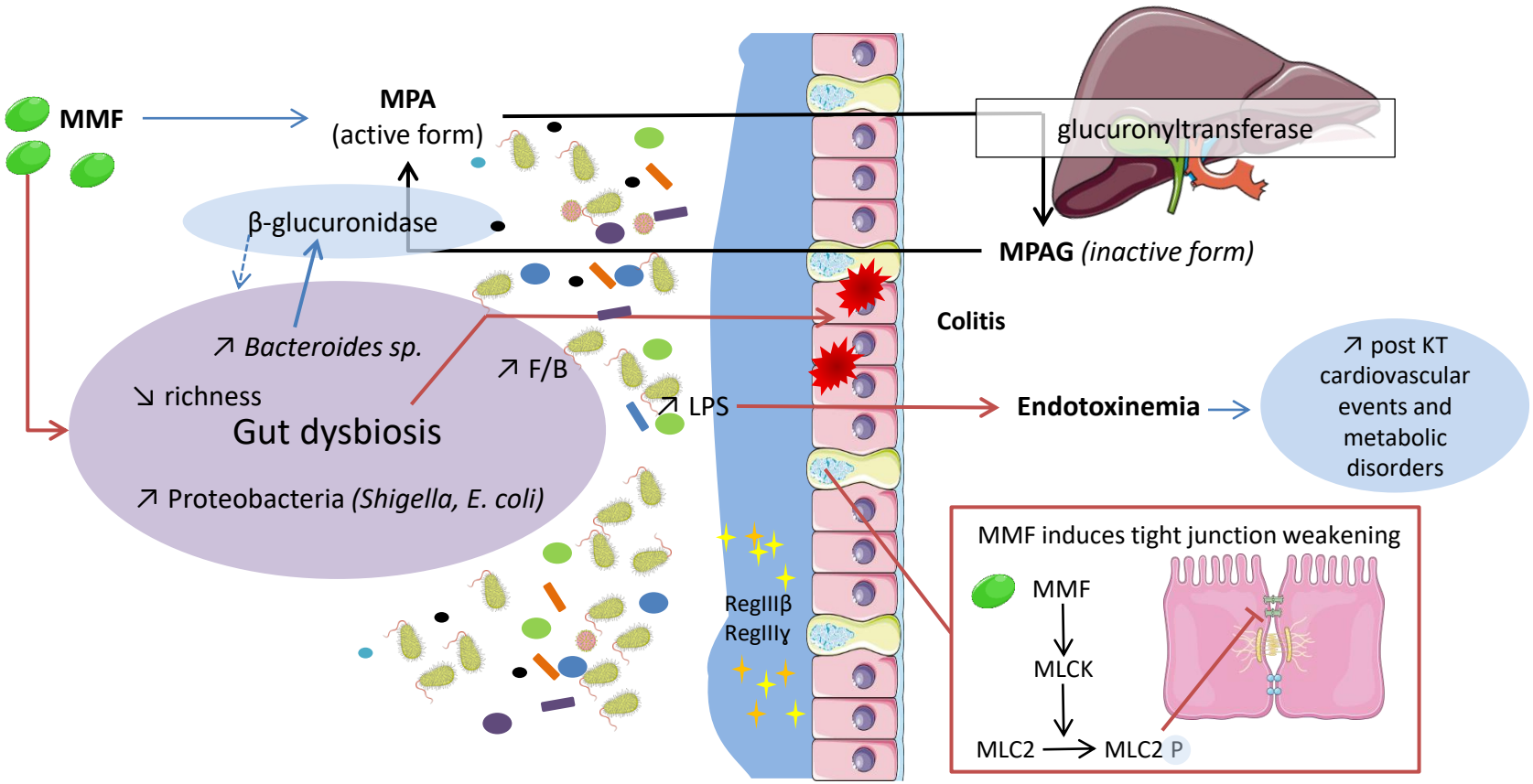
Chronic hemodialysis discontinuation

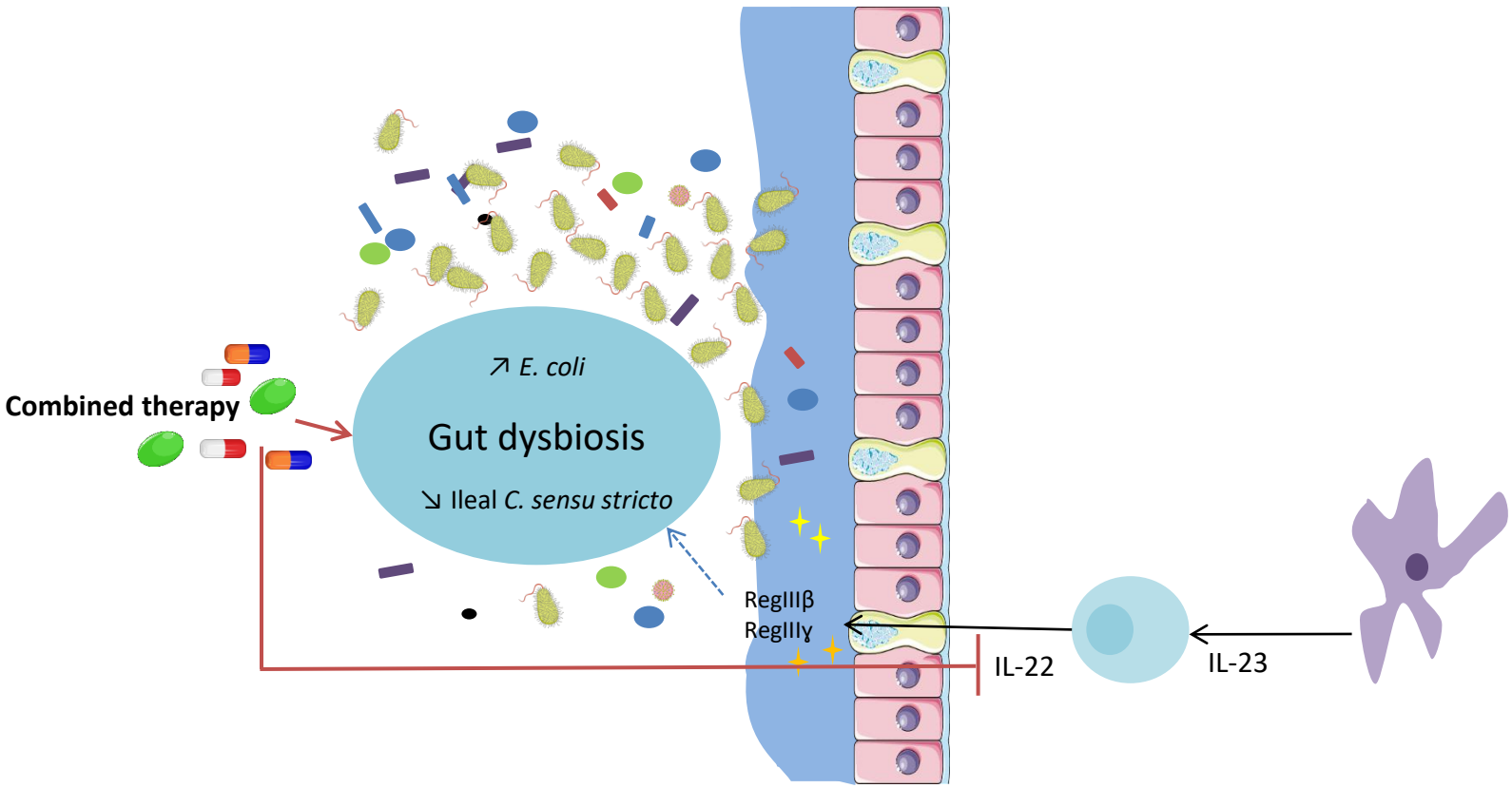
Lifestyle changes

Non-pharmacological interventions


Circadian clock
Muc2 expression
GCs reinforce tight junctions under inflammatory conditions
TNFα
MLCK
MLC2
MLC2 P
↘ Proteobacteria
Gut dysbiosis
↘ Mucispirillum
Delayed resilience
Glucocorticoids
C. scindens
Androgens
RegIIIβ
RegIIIγ
IL-22
IL-23
Increased severity of C. difficile infection


Microbiota functional alterations
↘ Oscillospira
Gut dysbiosis
↘ SCFA producing bacteria
Tacrolimus
M1
F. prausnitzii
Clostridiales
Muc3 expression
RegIIIβ
IL-22
IL-23
Colonic Treg
Systemic Treg
FK beneficial effects
Immunosuppression
FK sides effects
diabetes
High blood pressure


MMF
MPA
(active form)
glucuronyltransferase
β-glucuronidase
MPAG (inactive form)
↗ Bacteroides sp.
↘ richness
Gut dysbiosis
↗ Proteobacteria (Shigella, E. coli)
↗ F/B
↗ LPS
Colitis
Endotoxinemia
↗ post KT cardiovascular events and metabolic disorders
RegIIIβ
RegIIIγ
MMF induces tight junction weakening
MMF
MLCK
MLC2
MLC2 P


Combined therapy
↗ E. coli
Gut dysbiosis
↘ Ileal C. sensu stricto
RegIIIβ
RegIIIγ
IL-22
IL-23

Legend

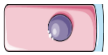
RegIII ɣ


RegIII β


Mucosal IgA


C. difficile


Opportunistic pathogenic bacteria


Intestinal epithelial cell


Mucus layer


Mechanism possibly involved in IS drug-induced dysbiosis


Inflammation